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(54) Title: PHOSPHATE DERIVATIVES OF VITAMIN E TO PROTECT CELLS FROM EFFECTS OF AGING AND INJURY (57) Abstract Phosphate-substituted vitamin E is effective for protecting cells from effects of aging and from chemically-induced cell injury and for stimulating cell repair. The phosphate ester of vitamin E is particularly useful for protecting the tissue in the intact animal when given in a protected form such as in microdroplets, microcrystals, or liposomes using protective materials such as lipids or lecithin.		

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PHOSPHATE DERIVATIVES OF VITAMIN E TO PROTECT CELLS FROM
EFFECTS OF AGING AND INJURY

Field of the Invention:

5 This invention relates to the protection of cells from effects of aging and injury by administration of phosphate-substituted vitamin E, particularly the vitamin E phosphate ester.

10 Background of the Invention:

A number of publications have discussed the merits of vitamin E in prevention of cell aging and toxic injury. The protective properties of vitamin E have been attributed to its role as a membrane-active antioxidant. It is believed that 15 vitamin E, a lipid soluble vitamin, dissolves in the phospholipid environment of the membranes and donates a hydrogen to terminate the free radical-induced peroxidation of the unsaturated fatty acids of membrane phospholipids. It has been generally accepted that it is by this mechanism that 20 vitamin E protects cells from free radical-induced injury.

There is no question that vitamin E is an essential dietary requirement, since vitamin E deficiency results in structural and functional alterations in various tissues such as liver, brain, heart, muscle, etc. As a result, vitamin E 25 has been used to treat various disorders of the heart, brain, liver and muscle. Unfortunately, vitamin E therapy has produced little or no benefit in most instances. This was not surprising, since results in cultures of hepatocytes suggest that vitamin E and vitamin E acetate (VEA) were relatively 30 inactive. Hence, it was seen that the administration of vitamin E as a medicinal was of minimal benefit.

The use of vitamin E to protect specifically against chemical-induced toxicity has been known. (Burton, et al, 35 "Vitamin E as an antioxidant in vitro and in vivo", Biology of vitamin E, Pitman, London (1983) London. Also see Yoshikawa

and Kondo, "Role of Vitamin E in the Prevention of Hepato-cellular Damage--", Vitamin E: Biochemical, Hematological, and Clinical Aspects, Lubin and Machlin, ed.; N.Y. Academy of Sci., (1982) 198-200.) Yoshikawa found no correlation between serum level of vitamin E and liver function, but did find a correlation between β -lipoprotein, a carrier of vitamin E, and liver function. Disturbance of liver function appears to arise, in such instances, from failure of effective delivery of vitamin E to the cell rather than as a result of host deficiency of vitamin E.

It has also been known that even when protection from cell injury is demonstrated using vitamin E in cell culture, a similar response often is not seen in the intact animal. The laboratory of Dr. Reed at Oregon State University has directed attention to the mechanism of protection against chemical-induced toxicity using vitamin E succinate. (See Pascoe, et al., Archives of Biochemistry and Biophysics, Vol. 253, No. 1, pp 150-158 and pp. 159-166 (1987).)

The need for a method of protecting liver cells from toxicity is particularly important because many medications are metabolized to toxic metabolites in the liver. A method which effectively protects the liver from medicinal-induced toxic injury would permit the use of medications that are toxic to liver tissue. An example of a compound that could be used to alleviate a disease condition but is toxic to liver tissue is tetrahydroaminoacridine (THA), a compound that has shown promise for use in treatment of Alzheimer's disease, but which is not currently in use because it has proven to be too hepatotoxic. It has been shown that vitamin E and vitamin E succinate are useful in protecting the liver from chemical-dependent damage in vitro. However, as discussed previously,

vitamin E has been found to be less useful in vivo in providing protection of the liver. (See Dogterom, et al, Biochemical Pharmacology, Vol 37, No 12, pp. 3211-2313 (1988).)

Attempts have been made to improve in vivo response by esterification of vitamin E. The most commonly used vitamin

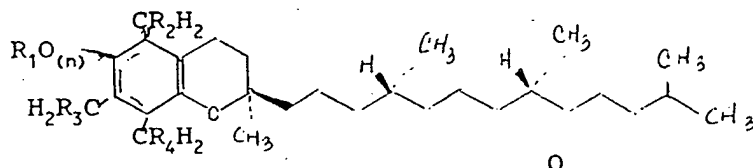
E esters are the acetate (VEA) and the succinate (VES) esters. (Fariss, et al, Toxicology Letters, 47 (1989) 61-75). Fariss' findings indicate that vitamin E succinate is superior to vitamin E and VEA in providing protection of cells from toxicant injury. However, the degree of protection seen in the cell cultures has not been reflected in protection of tissues in the intact animal.

The delivery of active agents to the site where beneficial effect is needed presents several problems. Many biologically active agents are quickly destroyed in the body by enzymes before they reach their intended target tissue. Furthermore, some drugs are unable to cross membrane barriers. The 'packaging' of pharmaceutically active agents to avoid destruction in the body's environment and to effectively deliver active agents across membrane barriers has, for many years, been accomplished by the use of liposomes, microdroplets, and microcrystals. Liposomes consist of phospholipid vesicles containing water-soluble drugs (See, for example, U.S. Patent 4,241,046, which is incorporated herein by reference). They consist of a spherical lipid bilayer with an aqueous phase inside. Other preparations such as microdroplets (See U.S. Patent 4,725,442, which is incorporated herein by reference) and microcrystals (See Patent Publication WO 91/16068) have also been used. The delivery of the vitamin E phosphates, as disclosed herein may advantageously be administered in any of the above formulations.

Disclosure of the Invention:

It has now been found that phosphate-substituted vitamin E is effective for protecting cells from effects of aging and from chemically-induced cell injury and for stimulating cell repair. The phosphate ester of vitamin E is particularly useful for protecting the tissue in the intact animal when given in a protected form such as in microdroplets, microcrystals, or liposomes using protective materials such as lipids or lecithin. A preferred route of administration for effecting protection of liver tissue is intraperitoneal

injection or infusion. The form of the vitamin E phosphate medicinal composition and route of administration of VEP will depend on the target organ. There are several positions on the vitamin E molecule that could be phosphorylated. Phosphated vitamin E compounds include:



wherein R_1 , R_2 , R_3 and R_4 are H or $O=P(=O)(O^-)_n$ and n is 0 or 1 with the proviso that at least one R is not hydrogen.

The vitamin E phosphate ester (VEP) was tested against vitamin E succinate (VES) in cell culture and was found to be four times as active as vitamin E succinate. Vitamin E and vitamin E acetate (VEA) were inactive under the same conditions. Because the growth of liver cells in tissue culture is very useful for research, for diagnostic purposes and for production of products of the liver *in vitro*, the use of the vitamin E phosphate in tissue culture is also an important embodiment of this invention.

Materials and Methods:

The vitamin E phosphate ester was obtained from Sigma.

Media used to grow hepatocytes was made using the following method:

Waymouth 752/1 amino acids (without valine) (A.A. Mix)

<u>Amino Acid</u>	<u>gm/50 liters</u>
L-Aspartic Acid	3.0 gm
L-Cystine	0.75 gm
L-Glutamic Acid	7.5 gm
Glycine	2.5 gm
L-Isoleucine	1.25 gm
L-Leucine	2.5 gm
L-Proline	2.5 gm
L-Threonine	3.75 gm
L-Tyrosine	2.0 gm
Cysteine	3.05 gm
L-Histidine	6.4 gm
L-Lysine	12.0 gm
L-Tryptophan	2.0 gm
L-Methionine	2.5 gm
L-Phenylalanine	2.5 gm
L-Ornithine	1.0 gm

The amino acids were mixed well with mortar and pestle.
Mixture was stored at room temperature in a dark bottle.

Monolayer Supplement (#1)

		mg/ml		mg/ml
5	Glucagon	0.71	B-Estradiol	0.75
	Testosterone	0.79	Dexamethasone	0.79
	Oleate	5	Linoleate	5

This mixture of hormones and fatty acids is made and stored frozen in 0.5 ml aliquots.

10 Monolayer Supplement #2

A mixture of aminolevulinic acid, 1.7 mg/ml and selenium, 0.017 mg/ml is stored frozen in 0.1 ml aliquots.

Waymouth 752/1 media: Preparation of 10 liters

15	H ₂ O	7 liters	AA mix	11.15 g
	HCl	1.515 g	KH ₂ PO ₄	0.808 g
	MgSO ₄	0.9899 g	NaCl	69.39 g
	Na ₂ HPO ₄	8.989 g	CaCl ₂ ·H ₂ O	1.212 g
	MgCl ₂ ·6H ₂ O	2.424 g	NaHCO ₃	7.07 g

20 Adjust pH to 7.40, adjust volume to 10 L. having water osmolarity of 280-295.

Hepatocyte Culture Medium:

To 500 ml. Waymouth 752/1 media add:

- 0.5 ml aliquot of monolayer supplement #1
- 25 0.1 ml aliquot monolayer supplement #2
- 5 ml Glutamine stock solution (17.5 g/500 ml)
- 5 ml glucose stock solution (180 g/500 ml)
- 5 ml valine stock solution (3.25 g/500 ml, pH 7.4)
- 5 ml Penicillin stock solution (5,000,000 units/500 ml)
- 30 5 ml gentamicin stock solution (5 mg/ml)
- 5 ml Vitamin C stock solution (5 mg/ml)
- 1.25 ml insulin (100 units/ml.)
- 10 ml pyruvic acid stock solution (11.22 g/100 ml)

Example 1:

- 35 Hepatocyte monolayers were incubated in hepatocyte culture medium described above in the presence of several different agents that have proven toxic to cell function, including ethanol (100 mM), t-butyl hydroperoxide (TBH) (0.5 mM), cocaine (0.5 mM), allyl alcohol (0.5 mM), CdCl₂ (10 μM), CCl₄ (0.5 mM), and BrCCl₃ (0.1 mM). In all in-
- 40

stances the following results were seen:

- 1) Phosphatidylcholine hydrolysis increased and was maintained during the incubation.
- 2) Reversible membrane injury was evidenced when phosphatidylcholine (PC) biosynthesis and hydrolysis were increased in a corresponding fashion.
- 3) Irreversible membrane injury, as evidenced by PC hydrolysis that exceeded the rate of PC biosynthesis was seen when PC biosynthesis was significantly depressed.

Example 2:

Monolayers of hepatocytes were incubated in the medium described above in the presence of 100 μ M vitamin E esters: vitamin E acetate (VEA), vitamin E succinate (VES), and vitamin E phosphate ester (VEP). The effect on PCT (Phosphocholine cytidyltransferase) activity and 3H-choline incorporation into PC is shown below as % of control \pm SEM

	Control	VEA	VES	VEP
3H-choline \rightarrow PC	100% \pm 5	110% \pm 13	221% \pm 15*	700% \pm 24*
PCT activity	100% \pm 2	95% \pm 3	185% \pm 5*	641% \pm 11*

* $P \leq .01$ significance from control. SEM = Standard error of the mean.

Example 3:

The dose dependent changes in incorporation of 3H-choline into PC of hepatocyte monolayers was evaluated after a 24 hour incubation period using varying doses of VEP and VES. The following results were observed:

Percent of control \pm SEM 3H-choline \rightarrow PC (Control= 100% \pm 3%)

	50 μ m	100 μ M	250 μ M	500 μ M
VES	135 \pm 8	175% \pm 11*	275% \pm 21*	350% \pm 29*
VEP	385 \pm 15*	590% \pm 34*	631% \pm 38*	550% \pm 22*

* $P \leq 0.01$ significance from control.

Example 4:

The ability to reduce the adverse effects of several toxins on cells incubated with the toxic agents for 24 hours was evaluated. Ability to reduce chemical-induced cell injury was studied comparing VEA, VES and VEP with controls in accord with the method described above.

Percent of control \pm SEM 3H-choline \rightarrow PC

Toxic Agent	Control	VEA	VES	VEP
.5 mM CCl_4	21 \pm 2	30 \pm 2	38 \pm 4	147 \pm 13
.1 mM BrCCl_3	14 \pm 1	32 \pm 3	64 \pm 4	187 \pm 35
.25 mM allyl alcohol	10 \pm 2	33 \pm 4	56 \pm 3	206 \pm 16
5 μ M CdCl_2	7 \pm 1	30 \pm 2	57 \pm 4	135 \pm 5

All data was significant to $P \leq 0.01$.

Example 5:

In a test to evaluate ability to stimulate cellular repair functions, aged or chemically exposed hepatocytes were cultured. After cellular damage was observed, the cells were then incubated with VEP for 24 hours. As can be seen from the data, when the cells were not irreversibly injured, VEP markedly stimulated cell function.

Percent of control \pm SEM 3H-choline \rightarrow PC (Control = 100%)

Agent:	Control	Cocaine	CCl_4	BrCCl_3	TBH	Aging
	100 \pm 6	166 \pm 4	206 \pm 12	176 \pm 13	147 \pm 2	331 \pm 18

Significant for all numbers at $P \leq 0.01$.

The data clearly indicated that VEP is able to reverse cell dysfunction in moderately damaged cells.

Example 6:

Vitamin E phosphate was administered intraperitoneally to rats as microcrystals formulated using the calcium salt of the vitamin E phosphate according to the method of Haynes (See Patent Publication WO) by sonication with egg lecithin. The composition was administered in conjunction with a dose (60mg/Kg) of allyl alcohol that caused lethal liver injury.

The vitamin E phosphate was prepared as a calcium salt to decrease water solubility and was formulated into microcrystals. The formulation containing 10% vitamin E phosphate (VEP) was administered intraperitoneally to deliver a dosage of VEP of 50mg/Kg. Fifteen minutes after administration of the VEP allyl alcohol was administered by intraperitoneal injection at the dosage of 60 mg/Kg. (See Example 8 as a method of preparation.)

All of the control animals exposed to the allyl alco-

hol showed markedly elevated levels (9-fold) of SGPT (indicator of liver injury) within 12 to 16 hours after exposure to the alcohol. After 18 hours all of the alcohol-exposed animals who had not received VEP had died. The animals who had received VEP showed only a 2-fold increase in SGPT. All of the VEP-protected animals survived.

The results clearly indicate that VEP protected the liver from toxic effects of the alcohol-induced liver cell damage in vivo.

Example 7:

Liposomes containing the calcium salt were prepared. Phosphatidylcholine (200 mg) was dissolved in 5 ml of DMSO. 200 mg of the vitamin E phosphate (calcium salt) was added. The mixture was sonicated 5 min. at 37°C degrees. Fifteen ml of 0.9% saline was added. The mixture was then sonicated for 15-30 minutes at 37°C.

Example 8:

A composition of microcrystals is prepared using 2 gm. of the calcium salt of the vitamin E phosphate to which is added 8 gm lecithin in 40 ml of isotonic glucose solution. The mixture is sonicated for 30 minutes and allowed to concentrate to 20% by sedimentation overnight. The preparation is then centrifuged in a clinical centrifuge at medium speed for 15 minutes. The precipitate is separated and resuspended in 10 ml of isotonic glucose and washed.

Example 9:

Influence of VEP liposomes on Allyl alcohol-induced liver injury was evaluated in male albino mice.

Treatment	SGPT
	% of control \pm SEM
Control (9) vehicle only	100 \pm 7
Allyl alcohol (9)	330 \pm 15 **
Vehicle plus VEP (9)	100 \pm 3 **
Allyl alcohol plus VEP (9)	109 \pm 4 **

** The mice were exposed to a single intraperitoneal dose of allyl alcohol (50 mg/kg) or vehicle for 4 hours.

Example 10:

Compositions are prepared using the sodium salt of the vitamin E phosphate as obtained from Sigma. The material is prepared by sonication of the salt of vitamin E phosphate in saline with lecithin to provide liposomes by the method of Example 7.

The use of vitamin E as an agent to protect cells from toxic injury has shown little or no promise for use as a therapeutic in vivo. It is now seen that the phosphate ester of the vitamin, when formulated in a manner that prevents hydrolysis by esterases in the gut and serum, can be used to protect cells from toxic injury in vivo. When treating the intact animal, any technology that delivers the phosphate ester of the vitamin E to the tissues subject to damage from aging or exposure to toxin will be appropriate. The use of liposome technologies to protect the active agents provides a useful means of delivery. Likewise, the microdroplets or microcrystals can be used.

Example 11:

Additional data was obtained comparing the effect of various concentrations of vitamin E (VE) and vitamin E phosphate (VEP) on the TBH-induced rise in hepatocellular lipid peroxidation. The following data was obtained:

Addition	nmoles MDA/mg protein +/- SEM
none	0.058 +/- 0.003
1 mM TBH	1.860 +/- 0.080
1 mM TBH + 50 μ M VE	1.750 +/- 0.020
1 mM TBH + 100 μ M VE	1.650 +/- 0.030
1 mM TBH + 250 μ M VE	1.410 +/- 0.020
1 mM TBH + 50 μ M VEP	1.260 +/- 0.010*
1 mM TBH + 100 μ M VEP	0.960 +/- 0.010*
1 mM TBH + 250 μ M VEP	0.430 +/- 0.010*

* Level of significance from VE preparations: $p \leq 0.01$

Example 12:

It has further been shown that VEP markedly stimulates membrane repair processes (PC biosynthesis) in cultured hepatocytes at all time periods studied (See table below.) Therefore, VEP can reverse cell aging and chemical-induced

cell injury by stimulating the cell's ability to repair injured membranes. Alterations in the incorporation of 3H-choline into PC was studied in cultured hepatocytes which were incubated 24 to 96 hours with (+) and without (-) 25 μ M VEP. The findings were as follows:

Time +/- VEP	nmoles 3H-choline-PC/min/mg protein +/- SEM
24 h (-)	33.8 \pm 1.0
24 h (+)	95.5 \pm 6.3*
48 h (-)	22.3 \pm 0.9
48 h (+)	99.4 \pm 5.2*
72 h (-)	29.2 \pm 2.4
72 h (+)	252.1 \pm 3.7*
96 h (-)	30.4 \pm 3.7
96 h (+)	340.3 \pm 6.2*

* Level of significance from control (-VEP) is $p \leq 0.01$.

As indicated in the examples, the use of vitamin E and its acetate ester does not provide benefit in vivo and its effectiveness on cultured hepatocytes was minimal, while the effect of the vitamin E succinate and vitamin E phosphate esters was beneficial. However, the phosphate was, surprisingly, far superior to previously tested esters. Therefore, it appears that the structural means by which vitamin E is presented to the cell is very important in altering cell dysfunction.

The vitamin E phosphates can, in accord with the teachings herein, be added to solutions used for storage and transport of tissues for transplant. One of the major problems in the transport of organs is the damage to cells between the time the organ is harvested and the time the organ is connected to the recipient's blood supply. The use of vitamin E phosphate to prevent tissue damage could greatly assist in improving the efficacy of such transplants. The concentration of the vitamin E phosphates can vary greatly. For example, concentrations of 1 μ M to 1000 μ M would be appropriate. A preferred concentration is 10 μ M to 100 μ M. The vitamin E phosphate can be added as one of the

soluble salts such as the sodium or potassium salts. An isotonic solution could be prepared containing the ester which could be in the form of a salt. If serum is added to the solution, the ester might be added one of the lipophilic protected forms such as those cited herein. The use of VEP as an additive to such to solution for storage and transport would be useful with any tissue for transplant, such as heart, liver, muscle (including heart muscle), lung, kidney tissue.

The particular method used to deliver compositions of the invention to the tissues of the intact animal will depend on the particular tissue to which it is administered. If the compositions will, while reaching the site of action, be exposed to esterases, the phosphate may, for example, be encapsulated in a protective coating for delivery to the target organ. Hence, liposomes, inclusion complexes such as cyclodextrin, microcrystals, or microdroplets may be used as means of delivery to the target organ.

The vitamin E phosphate may be delivered to the heart muscle by any means that will deliver the VEP to the heart tissue, including by intravenous injection (preferably using a protected form of the compound) or by infusion into the heart muscle.

Compositions containing the vitamin E phosphate can be delivered as mists or aerosols to the respiratory system or directly to tissues during surgery. They may be infused into tissues during or following transplant or surgery in an isotonic solution such as normal saline. The compositions of the invention can be delivered in drops (for example, as eye drops) or as infusions to the target tissues.

Compositions of vitamin E phosphate can be administered to the skin as creams, gels, or liquids. The protective VEP may be administered to minimize skin damage and could be added to cosmetics to prevent damage from the aging process. When the vitamin E phosphate is applied to

tissue that has been burned or abraded, the application of the composition as a sterile isotonic solution may be appropriate.

5 The compositions of the invention can also be administered as suppositories or in depo agents.

Compositions of the invention can be administered intrathecally to facilitate contact of the active agent with neuronal tissue after head injury. However, the VEP may also be administered intravenously. During brain
10 surgery, it could be administered directly to the brain tissue.

The compositions of the invention may be delivered in any protected form known in the art such as liposomes, microdroplets, microcrystals, cyclodextrin inclusion complexes, etc.
15

One theory that could account for the beneficial effect of VEP is that the phosphate ester markedly increases the ability of vitamin E to partition in the phospholipid bilayer of the membrane.

20 It is believed that the cellular toxicity of most drugs and chemicals is associated with "oxidative stress" and a rise in membrane-bound free radicals. Free radicals can alter the structure and function of cellular components by rapidly interacting with such components in the cell as
25 unsaturated or sulfur-containing amino acids, nucleic acids, and unsaturated fatty acids of phospholipids.

CLAIMS

1. A pharmaceutical effective composition of matter comprising a cell-protecting effective amount of vitamin E phosphate or a salt thereof in a lipophilic protective carrier.
2. A composition of claim 1 wherein the vitamin E phosphate is present in the form of liposomes, microcrystals, microdroplets or cyclodextrin inclusion complexes.
3. A sterile composition of claim 1 in a closed container.
4. A composition of claim 3 wherein the closed container is a vial.
5. A method of protecting cells from dysfunction arising from aging or injury comprising contacting the cells with a cell-protecting effective amount of a composition of claim 1.
6. A method of claim 5 wherein the cells are in an intact animal.
7. A method of claim 5 wherein the cells are in a tissue being preserved for transplant.
8. A method of claim 5 wherein the cells are in a tissue culture.
9. A method of claim 5 wherein the vitamin E phosphate ester is administered to an animal intraperitoneally.
10. A method of claim 5 wherein the vitamin E phosphate ester is delivered as a mist.
11. A method of claim 5 wherein the vitamin E phosphate is administered in liposomes, microcrystals, or microdroplets.
12. A method of claim 5 wherein the vitamin E phosphate is administered intravenously.
13. A method of claim 5 wherein the vitamin E phosphate is administered intrathecally.
14. A method of claim 5 wherein the vitamin E phosphate is administered as a cyclodextrin inclusion complex.
15. A method of claim 5 wherein the vitamin E phosphate is

administered by injection into the heart muscle.

16. A method of claim 5 wherein the vitamin E phosphate is administered to the tissue during surgery.
17. A method of claim 5 wherein the vitamin E phosphate is administered as drops.
18. A method of claim 5 wherein the cells are neuronal tissue cells.
19. A method of claim 5 wherein the cells are liver tissue cells.
20. A method of claim 5 wherein the cells are lung tissue cells.
21. A method of 5 wherein the cells are kidney tissue cells.
22. A method of claim 5 wherein the cells are heart tissue cells.
23. A method of claim 5 wherein the tissues are epidermal tissues.
24. A method of claim 23 wherein the vitamin E phosphate ester is administered as a cosmetic.
25. A composition of matter comprising a serum-free cell culture medium containing vitamin E phosphate and cell nutrients.
26. A composition of matter of claim 25 containing, additionally, hepatocytes.
27. A method of growing cells in cell culture by culturing the cells in serum-free cell growth media containing vitamin E phosphate.
28. A method of claim 5 wherein the tissues are gastrointestinal tissues.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01188

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/355

US CL :514/458

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	International Publication WO91/16068 (HAYNES ET AL.) 31 October 1991, See entire document.	1-28
Y	Chemical Abstracts, Vol. 107, #83908d, issued 1987, (TRUIA ET AL.) "Drug for Increasing Cellular Immunity, Useful in the Treatment of Viral Diseases and Cancer" See entire document, Rom. R089761.	1-28
Y	Chemical Abstracts Vol. 114, #178353c, issued 1991, (VERBOLOVIDH ET AL.) "Effect of α -Tocopherol in Water-soluble Form on Human Erythrocyte Membrane Interaction with Oxygen", See entire document, Biol. Nauki (USSR).	1-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search

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Date of mailing of the international search report

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